

CLAIMS

What is claimed is:

- 1 1. A substantially purified translocated intimin receptor (Tir) polypeptide that binds intimin.
- 1 2. The polypeptide of claim 1, wherein the unphosphorylated polypeptide has a molecular weight of about 78 kilodaltons as determined by SDS-PAGE under reducing conditions.
- 1 3. The polypeptide of claim 1, wherein the polypeptide is secreted by an attaching and effacing (A/E) pathogen.
- 1 4. The polypeptide of claim 3, wherein the polypeptide is secreted by enteropathogenic *E. coli*.
- 1 5. The polypeptide of claim 3, wherein the polypeptide is secreted by enterohemorrhagic *E. coli*.
- 1 6. The polypeptide of claim 1, wherein the polypeptide has an amino acid sequence as set forth in SEQ ID NO:2.
- 1 7. The polypeptide of claim 1, wherein the polypeptide has an amino acid sequence as set forth in SEQ ID NO:4.
- 1 8. An isolated polynucleotide encoding the polypeptide of claim 1.
- 1 9. The polynucleotide of claim 8, wherein the sequence encodes the amino acid sequence set forth in SEQ ID NO:2.

1 10. The polynucleotide of claim 8, wherein the sequence encodes the amino acid sequence
2 set forth in SEQ ID NO:4.

1 11. A polynucleotide selected from the group consisting of:
2 a) SEQ ID NO:1;
3 b) SEQ ID NO:1, wherein T is U;
4 c) nucleic acid sequences complementary to a) or b); and
5 d) fragments of a), b), or c) that are at least 15 nucleotide bases in length and that
6 hybridize to DNA which encodes the polypeptide set forth in SEQ ID NO:2.

1 12. A polynucleotide selected from the group consisting of:
2 a) SEQ ID NO: 3;
3 b) SEQ ID NO: 3, wherein T is U;
4 c) nucleic acid sequences complementary to a) or b); and
5 d) fragments of a), b), or c) that are at least 15 nucleotide bases in length and that
6 hybridize to DNA which encodes the polypeptide set forth in SEQ ID NO: 4.

1 13. A vector containing the polynucleotide of claim 8.

1 14. A host cell containing the vector of claim 13.

1 15. An anti-Tir antibody which binds to the polypeptide of claim 1.

1 16. The antibody of claim 15, wherein the antibody is monoclonal.

1 17. The antibody of claim 15, wherein the antibody is polyclonal.

- 1 18. A method for detecting Tir polypeptide in a sample, comprising:
- 2 a) contacting the sample with the antibody of claim 15; and
- 3 b) detecting binding of the antibody to Tir polypeptide, wherein binding is indicative
- 4 of the presence of Tir polypeptide in the sample.
- 1 19. The method of claim 18, wherein the sample is tissue.
- 1 20. The method of claim 18, wherein the sample is a biological fluid.
- 1 21. The method of claim 18, wherein the presence of Tir polypeptide in the sample is
2 indicative of infection by enteropathogenic *E. coli*.
- 1 22. The method of claim 18, wherein the presence of Tir polypeptide in the sample is
2 indicative of infection by enterohemorrhagic *E. coli*.
- 1 23. A method of ameliorating disease caused by Tir-producing organism, comprising:
2 inducing an immune response in a host with the polypeptide of claim 1, thereby
3 ameliorating disease caused by infection of the host by the Tir-producing organism.
- 1 24. The method of claim 23, wherein the host is human.
- 1 25. The method of claim 23, wherein the host is bovine.
- 1 26. The method of claim 23, wherein the Tir-producing organism is *E. coli*.
- 1 27. The method of claim 23, wherein the Tir-producing *E. coli*. is an enteropathogenic *E.*
2 *coli*.
- 1 28. The method of claim 23, wherein the Tir-producing *E. coli*. is an enterohemorrhagic *E.*
2 *coli*.

- 1 29. A method for detecting *tir* polynucleotide in a sample, comprising:
- 2 a) contacting a sample suspected of containing *tir* polynucleotide with a nucleic acid
3 probe that hybridizes to *tir* polynucleotide; and
- 4 b) detecting hybridization of the probe with *tir* polynucleotide, wherein the detection
5 of hybridization is indicative of *tir* polynucleotide in the sample.
- 1 30. The method of claim 29, wherein the nucleic acid probe is selected from the group
2 consisting of:
- 3 a) a nucleic acid sequence set forth in SEQ ID NO:1;
- 4 b) a nucleic acid sequence set forth in SEQ ID NO:1, wherein T is U;
- 5 c) a nucleic acid sequence complementary to a) or b); and
- 6 d) fragments of a), b), or c) that are at least 15 nucleotide bases in length and that
7 hybridize under stringent conditions to DNA which encodes the polypeptide set
8 forth in SEQ ID NO:2.
- 1 31. The method of claim 29, wherein the nucleic acid probe is selected from the group
2 consisting of:
- 3 a) a nucleic acid sequence set forth in SEQ ID NO:3;
- 4 b) a nucleic acid sequence set forth in SEQ ID NO:3, wherein T is U;
- 5 c) a nucleic acid sequence complementary to a) or b); and
- 6 d) fragments of a), b), or c) that are at least 15 nucleotide bases in length and that
7 hybridize under stringent conditions to DNA which encodes the polypeptide set
8 forth in SEQ ID NO:4.
- 1 32. A method for detecting *tir* polynucleotide in a sample, comprising amplifying the *tir*
2 polynucleotide.

1 33. A recombinant method for producing *tir* polynucleotide, comprising
2 inserting a nucleic acid encoding a selectable marker into the polynucleotide of
3 claim 8, such that the resulting polynucleotide encodes a recombinant Tir
4 polypeptide containing the selectable marker.

1 34. A polynucleotide produced by the method of claim 33.

1 35. A host cell containing the polynucleotide of claim 34.

1 36. A recombinant method for producing Tir polypeptide, comprising:
2 a) growing a recombinant host cell containing a polynucleotide encoding Tir
3 polypeptide under conditions which allow expression and secretion of Tir
4 polypeptide; and
5 b) isolating the polypeptide.

1 37. A method of producing a Tir fusion protein comprising:
2 a) growing a host cell containing a polynucleotide encoding Tir operably linked to a
3 polynucleotide encoding a polypeptide or peptide of interest under conditions
4 which allow expression and secretion of the fusion protein; and
5 b) isolating the fusion protein.

1 38. A method of identifying a compound which interferes with the binding of a Tir
2 polypeptide to intimin, the method comprising
3 comparing the binding of the Tir polypeptide to intimin in the presence of the
4 compound to the binding of the Tir polypeptide in the absence of the compound.

- 1 39. A method for differentiating among attaching and effacing pathogens, comprising:
- 2 a) contacting the attaching and effacing bacteria with an antibody of claim 9; and
- 3 b) contacting the attaching and effacing bacteria with an anti-phosphotyrosine
- 4 antibody.
- 1 40. A method for delivering a compound of interest to a Tir-containing cell, comprising:
2 administering to the Tir-containing cell an intimin-containing cell delivery vehicle
3 which contains a compound of interest.
- 1 41. A method for detecting cytoskeleton of a cell, comprising:
2 a) contacting a cell cytoskeleton with Tir polypeptide; and
3 b) detecting the binding of Tir polypeptide to cell cytoskeleton.
- 1 42. A kit useful for the detection of Tir polypeptide comprising carrier means being
2 compartmentalized to receive in close confinement therein one or more containers
3 comprising a container containing an antibody which binds to Tir polypeptide.
- 1 43. The kit of claim 42, wherein the antibody is detectably labeled.
- 1 44. The kit of claim 43, wherein the label is selected from the group consisting of
2 radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent
3 compound, a metal chelate, and an enzyme.
- 1 45. A kit useful for the detection of a *tir* polynucleotide comprising carrier means being
2 compartmentalized to receive in close confinement therein one or more containers
3 comprising a container containing the nucleic acid probe that hybridizes to *tir*
4 polynucleotide.

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- 1 46. The kit of claim 45, wherein the probe is detectably labeled.
- 1 47. The kit of claim 46, wherein the label is selected from the group consisting of
2 radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent
3 compound, a metal chelate, and an enzyme.
- 1 48. A kit useful for the detection of a *tir* polynucleotide comprising carrier means being
2 compartmentalized to receive in close confinement therein two or more containers
3 comprising:
4 a) a first container containing a first nucleic acid probe that hybridizes to one of two
5 strands of *tir* polynucleotide; and
6 b) a second container containing a second nucleic acid probe that hybridizes to the
7 other of two strands of *tir* polynucleotide.
- 1 49. A method for inducing a cell mediated immune response to a polypeptide of interest,
2 comprising:
3 contacting a subject with an attenuated bacteria, wherein the bacteria lacks an EspA or
4 EspB protein and wherein the bacteria contains a polynucleotide encoding a fusion protein
5 comprising a Tir polypeptide operably linked to the polypeptide of interest.
- 1 50. The method of claim 49, wherein the polypeptide of interest is an antigen.
- 1 51. The method of claim 49, wherein the bacteria is *E.coli*.